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PRINCIPAL INVESTIGATOR: Farshid Guilak

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Steven A. Olson, Farshid Guilak, Virginia B. Kraus, Bridgette Furman, Kelly Kimmerling

E-Mail: guilak@duke.edu

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Duke University
School of Medicine
2200 West Main St.
Suite 820 Erwin Square Plaza
Durham, NC 27705

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14. ABSTRACT
The overall objective of this research effort is to identify biomarkers following articular fracture that may be predictive of the development of post-traumatic arthritis (PTA). PTA is a clinically important complication of joint injury with life-long effects for the patient. While PTA can occur rapidly after moderate to severe articular injuries, not every patient will go on to develop this condition. There are no effective screening methods to determine who is at risk. This proposal includes both a clinical observational study and a series of murine experiments, both with the goal of identifying biomarkers that are associated with development of PTA. Patients with knee joint fractures will be enrolled, and we will collect serum, urine, and synovial fluid early after injury. Radiographic imaging will be performed early after injury, again at 18 months, and analyzed to determine which patients developed PTA from those who did not. We will assess the ability of identified biomarkers in serum, urine, and synovial fluid to predict PTA following joint injury. Additionally, biomarkers will be assessed in a murine model of articular fracture using two strains in which one strain develops PTA and the other does not. Comparison of the human and mouse response to knee joint fracture will allow assessment of the potential use of the mouse model to evaluate future therapies to prevent PTA.

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"Assessment of Biomarkers Associated with Joint Injury and Subsequent Post-Traumatic Arthritis"
Start date: 9/30/2012
PIs – Steven A. Olson **(SAO)**; Farshid Guilak **(FG)**; and Virginia B Kraus **(VBK)**

1. INTRODUCTION:

Post-traumatic arthritis (PTA) is a clinically important complication of joint injury with life-long effects for the patient[1,2]. PTA is a severe burden in active duty and discharged soldiers[3]. While PTA can occur rapidly after moderate to severe articular injuries, not every patient will go on to develop this condition. There are no effective screening methods to determine who is at risk for developing PTA. The overall objective of this proposal is to identify biomarkers following articular fracture that may be predictive of the development of PTA. To accomplish this we will conduct a two-part study. We will perform a prospective observational study of patients with lower extremity articular fractures requiring operative treatment. Patients with knee joint fractures will be enrolled, and we will collect serum, urine, and synovial fluid from each patient acutely after injury. Radiographic imaging will be performed early after injury and again at 18 months. Both scans will be analyzed to separate the patients that developed PTA from those who did not. We will assess the ability of identified biomarkers in serum, urine, and synovial fluid to predict PTA following joint injury. Additionally, biomarkers will be assessed in a murine model of articular fracture using two strains in which one strain develops PTA and the other does not. Comparison of the human and mouse response to knee joint fracture will allow for assessment of the potential use of the mouse model to evaluate future therapies to prevent PTA. The low cost of mouse models lends itself to this type of work, and the results will provide a validated model to use for studying PTA. The goal of this work is to establish the basis for future use of biomarkers to predict the potential risk for developing PTA after acute joint injury. In addition this work will elucidate data on biospecimens that may be useful in future registries of acute joint injuries.

2. KEYWORDS:

Post-traumatic arthritis, post-traumatic osteoarthritis, articular fracture, joint injury, trauma, biomarker, inflammation, MRI, knee, mouse model, translational research.

3. OVERALL PROJECT SUMMARY:

The overall objective of this study is to identify biomarkers following articular fracture that may be predictive of the development of PTA. Specifically, patients with a closed unilateral articular fracture of the knee requiring operative treatment will be enrolled over an 18-month period. Biosamples (synovial fluid from the injured and contralateral uninjured knee, serum, and urine) will be collected prior to or at surgical intervention. MRI imaging of the injured knee will be obtained to assess the articular cartilage. Degenerative changes in the cartilage and joint space narrowing will be correlated to biomarkers that may be indicative and predictive of joint degeneration and the development of PTA. We have successfully enrolled patients, collected and stored biosamples, and have begun receiving MRI scans for study patients. Enrollment was initially slow. However, we addressed this issue by expanding the enrollment criteria. Sample collection and processing has been very successful, and we are pleased with the quantity of biosamples collected from each patient.

The second aim of this study is to create closed tibial plateau fractures in the left knee of C57BL/6 mice that develop PTA and MRL/MpJ mice that are protected from PTA. Serum and synovial fluid will be collected from both strains at various time points. Biospecimens will be analyzed for markers of joint inflammation and degradation identified in the human knee following articular injury. Biomarkers will be correlated to joint pathology that will be assessed from microCT and histology. The human and mouse biomarker profiles associated with PTA

will be compared to assess correlations between them. We have successfully completed the short-term data collection (pre-fracture, 0, 1, 7 and 14 days post-fracture), including receiving animals, fracturing, sacrificing, and collection of biosamples. MicroCT and histologic analyses have also been completed for the short-term cohort. We have successfully completed the long-term data collection (8 weeks post-fracture), including receiving the animals, fracturing, sacrificing, and collection of biosamples. MicroCT analyses are completed, and histologic analyses are beginning as of October 2014.

The details of our progress to date are described below with each task outlined from the approved Statement of Work (SOW).

A. SPECIFIC AIM 1: TASKS

Task 1. Review and approval of IRB protocol (months 1-4) **[SAO, VBK] COMPLETED**

- Duke IRB application submitted on 05/31/2012
- Duke IRB application approved on 07/11/2012
- Amendment to Duke IRB protocol to expand enrollment criteria submitted on 05/06/2013
- Amendment to Duke IRB protocol to expand enrollment criteria approved on 05/17/2013

Task 2. USAMRMC Office of Research Protections review and approval of human use documents (months 1-6) **[SAO] COMPLETED**

- IRB application submitted to USAMRMC Office of Research Protections for review on 09/06/2012
- IRB application approved by USAMRMC Office of Research Protections on 09/21/2012
- Request to expand enrollment criteria was submitted with prior progress report on 04/09/2013
- Response from USAMRMC ORP HRPO received on 05/02/2013.
    - We were informed that the expanded enrollment criteria was not a substantive modification/amendment to our protocol and does not increase risk to subjects. Therefore, the only action needed was to amend the protocol and submit to our Duke IRB for expedited review/approval.

Task 3. Enroll 30 patients in study (months 4-18) **[SAO, FG, VBK] COMPLETED**

3a. Patients with closed unilateral articular fracture of the knee requiring operative treatment will be enrolled in study

3b. Biosamples (synovial fluid, blood, urine) will be collected at time of placing a temporizing spanning external fixator

- First patient enrolled on 12/19/2012 **[SAO]**
    - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Second patient enrolled on 03/06/2013 **[SAO]**
    - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Third patient enrolled on 06/19/2013 **[SAO]**
    - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Fourth patient enrolled on 07/03/2013 **[SAO]**
    - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Fifth patient enrolled on 07/18/2013 **[SAO]**
    - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Sixth patient enrolled on 07/23/2013 **[SAO]**
    - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Seventh patient enrolled on 07/25/2013 **[SAO]**
    - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Eighth patient enrolled on 08/23/2013 **[SAO]**
    - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**

- Ninth patient enrolled on 09/12/2013 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Tenth patient enrolled on 10/29/2013 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Eleventh patient enrolled on 05/21/2014 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Twelfth patient enrolled on 05/29/2014 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Thirteenth patient enrolled on 06/05/2014 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Fourteenth patient enrolled on 06/10/2014 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Fifteenth patient enrolled on 06/25/2014 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Sixteenth patient enrolled on 06/19/2014 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Seventeenth patient enrolled on 09/04/2014 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Eighteenth patient enrolled on 09/25/2014 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**

*Task 4. V*isual analog pain score, the Knee injury and Osteoarthritis Outcome Score (KOOS), and the SF-36 will be completed within 2 weeks of injury. **[SAO] COMPLETED**

- Visual analog pain score, KOOS, and SF-36 for first patient was completed on 12/27/2012 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for second patient was completed on 03/06/13 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for third patient was completed on 06/20/2013 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for fourth patient was completed on 07/01/2013 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for fifth patient was completed on 07/17/2013 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for sixth patient was completed on 07/22/2013 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for seventh patient was completed on 07/23/2013 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for eighth patient was completed on 08/23/2013 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for ninth patient was completed on 09/13/2013 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for ninth patient was completed on 09/13/2013 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for tenth patient was completed on 10/31/2013 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for eleventh patient was completed on 05/20/2014 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for twelfth patient was completed on 05/21/2014 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for thirteenth patient was completed on 05/28/2014 **[SAO]**

- Visual analog pain score, KOOS, and SF-36 for fourteenth patient was completed on 06/09/2014 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for fifteenth patient was completed on 06/13/2014 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for sixteenth patient was completed on 06/20/2014 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for seventeenth patient was completed on 09/02/2014 **[SAO]**
- Visual analog pain score, KOOS, and SF-36 for eighteenth patient was completed on 09/22/2014 **[SAO]**

Task 5. Biosamples (synovial fluid, blood, urine) will be collected at time of definitive fixation of closed unilateral articular fracture of the knee requiring operative treatment. Timing of repair will be based on standard of care for treating the clinical injury. **[SAO, FG, VBK] COMPLETED**

- Biosamples from first patient collected at time of definitive fixation on 12/22/2012 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- Biosamples from second patient collected at time of definitive fixation on 03/13/2013 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- The third patient enrolled had definitive fixation at the baseline visit on 06/19/2013 so a second set of biosamples was not collected.
- The fourth patient enrolled had definitive fixation at the baseline visit on 07/03/2013 so a second set of biosamples was not collected.
- The fifth patient enrolled had definitive fixation at the baseline visit on 07/18/2013 so a second set of biosamples was not collected.
- The sixth patient enrolled had definitive fixation at the baseline visit on 07/23/2013 so a second set of biosamples was not collected.
- The seventh patient enrolled had definitive fixation at the baseline visit on 07/25/2013 so a second set of biosamples was not collected.
- The eighth patient enrolled had definitive fixation at the baseline visit on 08/23/2013 so a second set of biosamples was not collected.
- The ninth patient enrolled had definitive fixation at the baseline visit on 09/12/2013 so a second set of biosamples was not collected.
- The tenth patient enrolled had definitive fixation at the baseline visit on 10/29/2013 so a second set of biosamples was not collected.
- The eleventh patient enrolled had definitive fixation at the baseline visit on 05/21/2014 so a second set of biosamples was not collected.
- The twelfth patient enrolled had definitive fixation at the baseline visit on 05/29/2014 so a second set of biosamples was not collected.
- The thirteenth patient enrolled had definitive fixation at the baseline visit on 06/05/2014 so a second set of biosamples was not collected.
- Biosamples from fourteenth patient collected at time of definitive fixation on 06/17/2014 **[SAO]**
  - Synovial fluid, blood, urine processed and stored in -80° freezer **[FG, VBK]**
- The fifteenth patient enrolled had definitive fixation at the baseline visit on 06/25/2014 so a second set of biosamples was not collected.
- The sixteenth patient enrolled had definitive fixation at the baseline visit on 06/19/2014 so a second set of biosamples was not collected.
- The seventeenth patient enrolled had definitive fixation at the baseline visit on 09/04/2014 so a second set of biosamples was not collected.
- The eighteenth patient enrolled had definitive fixation at the baseline visit on 09/25/2014 so a second set of biosamples was not collected.

Task 6. Post-operative follow-up of all patients (months 5-18) **[SAO]**
6a. Post-operative T1-rho MRI will be obtained
6b. Analysis of MRI T1-rho imaging of cartilage

- Post-operative MRI obtained from first patient on 01/30/2013 **[SAO]**
- Post-operative MRI obtained for second patient on 04/30/2013 **[SAO]**
- Post-operative MRI attempted for third patient on 08/06/2013; patient experienced claustrophobia associated with the MRI machine and may not return to study **[SAO]**
- Post-operative MRI not obtained for fourth patient because patient will have a total knee replacement; patient does not satisfy enrollment criteria and has been removed from the study **[SAO]**
- Post-operative MRI obtained for fifth patient on 11/01/2013 **[SAO]**
- Post-operative MRI obtained for sixth patient on 09/23/2013 **[SAO]**
- Post-operative MRI obtained for seventh patient on 09/26/2013 **[SAO]**
- Post-operative MRI not obtained for eighth patient because definitive fixation resulted in just an external fixation; patient does not satisfy enrollment criteria and has been removed from the study **[SAO]**
- Post-operative MRI obtained for ninth patient on 10/28/2013; patient withdrew from study so no further MRI will be obtained **[SAO]**
- Post-operative MRI obtained for tenth patient on 01/14/2014 **[SAO]**
- Post-operative MRI obtained for eleventh patient on 07/16/2014 **[SAO]**
- Post-operative MRI not obtained for twelfth patient; patient has been withdrawn from study due to non-compliance **[SAO]**
- Post-operative MRI not obtained for thirteenth patient; patient has been withdrawn from study due to non-compliance **[SAO]**
- Post-operative MRI obtained for fourteenth patient on 08/11/2014 **[SAO]**
- Post-operative MRI obtained for fifteenth patient on 08/15/2014 **[SAO]**
- Post-operative MRI obtained for sixteenth patient on 09/16/2014 **[SAO]**
- Post-operative MRI scheduled for seventeenth patient on 11/03/2014 **[SAO]**
- Post-operative MRI scheduled for eighteenth patient on 11/07/2014 **[SAO]**

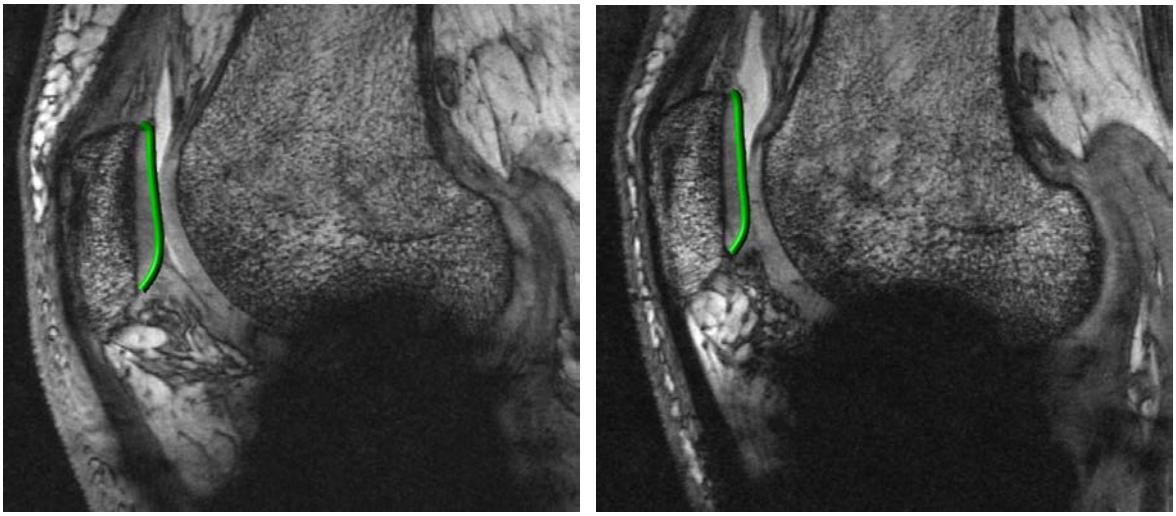
Task 7. 18-month follow-up of all patients (months 19-36) [SAO]

- 18-month follow-up MRI obtained for first patient on 07/10/2014 **[SAO]**
- 18-month follow-up MRI obtained for second patient on 09/24/2014 **[SAO]**
- 18-month follow-up MRIs scheduled for December 2014 for patients five, six, and seven **[SAO]**
- Analysis of post-operative MRIs and 18-month follow-up MRIs has begun **[SAO]**

B. SPECIFIC AIM 1: SUPPORTING DATA

For assessment of degenerative changes to joint tissues, post-operative (post-op) and 18-month follow-up MRI scans will be evaluated. MRI images are obtained using a using a double-echo steady state sequence (DESS). The outer margins of the patella and femoral bone cortices, as well as the surface of the articular cartilage, are outlined from the DESS MR images using solid-modeling software (Rhinoceros, Robert NcNeel and Associates, Seattle, WA)[4,5]. Next, each outline is placed in the appropriate spatial plane so that the curves can be compiled to generate 3D surface models of the patella and femur, and respective articular cartilage (Geomagic Studio, Research Triangle Park, NC). Cartilage thickness is then quantified by creating a grid of sampling points spanning the articular surface of the joint. Changes in cartilage thickness between post-operative and 18-month follow-up images will be quantified.

The effect of metal hardware used to fix the articular fracture is evident in the scan and obscures the tibial plateau articular surface (Figure 1, black shadow). However, the articular cartilage in the patella and femoral condyle is clearly visible at the patella-femoral joint. The articular cartilage surface of the patella is outlined in green on sagittal images from both the post-op and 18-month MRI scans in Figure 1.



Post-Operative MRI 18-Month MRI

Figure 1. Sagittal view of PTA002 MRI scans. Green outline indicates articular cartilage surface of the patella in both images.

C. SPECIFIC AIM 2: TASKS

Task 1. Review and approval of animal protocol (months 1-4) **[FG] COMPLETED**

- Duke IACUC application submitted on 07/02/2012
- Duke IACUC application approved on 07/25/2012
- Duke certification of IACUC Review and Approval/Grant concordance received on 08/21/2012
- Amendment to Duke IACUC to allow use of live microCT scanning submitted 06/10/2013.
- Amendment to Duke IACUC to allow use of live microCT scanning approved 07/17/2013.

Task 2. USAMRMC Office of Research Protections review and approval of animal use documents (months 1-4) **[FG] COMPLETED**

- ACURO animal use appendix submitted to USAMRMC Office of Research Protections for review on 08/29/2012
- Received ACURO approval letter on 11/29/2012
- Amendment to allow use of live microCT scanning submitted to ACURO 08/12/2013.
- Amendment to allow use of live microCT scanning approved to ACURO 08/20/2013.

Task 3. Obtain mice and create closed intra-articular fracture of the left knee of mice (months 5-9) **[FG] COMPLETED**

3a. Obtain C57BL/6 and MRL/MpJ mice at 8 weeks of age

- 60 mice were ordered on 01/07/2013 **[FG]**
  - 30 C57BL/6 mice were received at 9 weeks of age on 01/16/2013 **[FG]**
  - 30 MRL/MpJ mice were received at 10 weeks of age on 01/24/2013 **[FG]**
- Replacement C57BL/6 mice were ordered after receiving credit for 12 mice on 05/13/2013 **[FG]**

- 12 C57BL/6 mice were received at 10 weeks of age on 05/22/2013 **[FG]**
- 2 C57BL/6 mice died due to unidentified health reasons on 05/26/2013 **[FG]**
- 2 replacement C57BL/6 mice were received after credit on 08/07/2013 **[FG]**

3b. Allow mice to mature to 16 weeks of age

- Mice (30 MRL/MpJ and 18 C57BL/6) were housed until 03/04/2013 **[FG]**
- 10 C57BL/6 mice were housed until 07/17/2013 **[FG]**

3c. Create closed intra-articular fractures in the left knee of mice

- Fractures were created 03/05/2013 – 03/13/2013 **[FG]**
- Fractures (n=6) were created on 07/17/2013 **[FG]**

Task 4. Sacrifice mice and harvest samples for analyses (months 10-11) **[FG] COMPLETED**

4a. Sacrifice mice at pre-fracture, 0, 1, 7 and 14 days
4b. Collect serum and synovial fluid at time of sacrifice and store at -80°
4c. Harvest both hind limbs for analyses, store at -20°

- Mice were sacrificed (MRL/MpJ at pre-fracture, 0, 1, 7 and 14 days post-fracture and C57BL/6 at 0,1, and 7 days post-fracture) on 03/04/2013 – 03/27/2013 **[FG]**
  - Serum and synovial fluid were collected at time of sacrifice and stored at -80° **[FG, VBK]**
  - Both hind limbs were harvested at time of sacrifice and stored at -20° **[FG, VBK]**
- Mice were sacrificed (C57BL/6 at pre-fracture & 14 days post-fracture) on 07/31/2013 **[FG]**
  - Serum and synovial fluid were collected at time of sacrifice and stored at -80° **[FG, VBK]**
  - Both hind limbs were harvested at time of sacrifice and stored at -20° **[FG, VBK]**
- Mice were sacrificed (C57BL/6 at pre-fracture) on 08/08/2013. **[FG]**
  - Serum and synovial fluid were collected at time of sacrifice and stored at -80° **[FG, VBK]**
  - Both hind limbs were harvested at time of sacrifice and stored at -20° **[FG, VBK]**

Task 5. Perform microCT analyses on hind limbs (months 12-18) **[FG] COMPLETED**

- Limbs were scanned 09/04/2013-09/09/2013 **[FG]**
- Data processing and analysis complete for both hind legs **[FG]**

Task 6. Perform histologic analyses on hind limbs (months 18-24) **[FG] COMPLETED**

- Limbs were decalcified, dehydrated, and embedded in paraffin for histology **[FG]**
- Histologic grading and statistical analysis complete for both hind legs **[FG]**

Task 7. Obtain 24 additional mice and create closed intra-articular fracture of the left knee of mice (month 12-16) **[FG] COMPLETED**

7a. Obtain 12 C57BL/6 mice and 12 MRL/MpJ mice at 8 weeks of age

- 24 mice were ordered on 07/29/2013 **[FG]**
  - 12 C57BL/6 mice were received at 9 weeks of age on 08/07/2013
  - 12 MRL/MpJ mice were received at 8 weeks of age on 08/15/2013
  - 2 C57BL/6 mice died due to unidentified health reasons on 08/13/2013 **[FG]**
  - 2 replacement C57BL/6 mice were received after credit on 08/28/2013 **[FG]**

7b. Allow mice to mature to 16 weeks of age **[FG]**
7c. Create closed intra-articular fractures in the left knee of mice **[FG]**

Task 8. Sacrifice mice and harvest samples for analyses (months 17-18) **[FG, VBK] COMPLETED**

8a. C57BL/6 mice and MRL/MpJ mice were sacrificed at 56 days post-fracture **[FG]**
8b. Collected serum and synovial fluid at time of sacrifice and stored at -80° **[FG, VBK]**

8c. Both hind limbs were harvested and stored at -20° **[FG]**

Task 9. Perform microCT analyses on hind limbs (months 19-24) **[FG] COMPLETED**
- Limbs were scanned ex-vivo **[FG]**
- Data processing and analyses complete for both hind legs **[FG]**

Task 10. Perform histologic analyses on hind limbs (months 25-32) **[FG]**
- In progress as of October 2014 **[FG]**

D. SPECIFIC AIM 2: SUPPORTING DATA

Bone Morphological Changes Correlate with Reduction in PTA after Articular Fracture in the MRL/MpJ Mouse

The objective was to identify differences in acute joint pathology and degeneration in the articular cartilage as well as other joint tissues, including the synovium and periarticular bone following articular fracture in C57BL/6 and MRL/MpJ mice.

Six mice from each strain (C57BL/6 and MRL/MpJ) did not receive a fracture and served as pre-fracture controls. Mice were sacrificed at 0, 1, 7, 14, and 56 days after fracture (n=6-11 per strain per time point). The left (fractured) and right (non-fractured) limbs were harvested, formalin fixed and scanned with microCT to assess bone morphology in the tibial epiphysis and metaphysis and femoral condyles. Histology sections (FFPE, 8μm thick in coronal plane) of all limbs were assessed for cartilage degeneration in the lateral and medial femoral condyles (LF, MF) and lateral and medial aspects of the tibial plateau (LT, MT) using a modified Mankin score, synovial inflammation using a modified synovitis score with semi-quantitative scales, and osteophyte score[6-10]. Parametric analyses were performed for bone morphological measures and histological assessment.

Subchondral bone thickening was significantly increased in the C57BL/6 mice compared to the MRL/MpJ mice in the medial femur ($p=0.03$) and the medial tibia ($p=0.01$), but not on the lateral side. Bone morphological changes in response to fracture were significantly different between the two mouse strains. In the fractured limbs, bone mineral density (BMD), bone volume (BV), and bone fraction (BV/TV) in both the tibial epiphysis and metaphysis were significantly greater ($p<0.002$) in the MRL/MpJ strain compared to the C57BL/6 strain ($p<0.001$). However, in the femoral condyles, both BMD and cancellous bone fraction (BV/TV) were significantly increased in the C57BL/6 strain compared to the MRL/MpJ strain ($p=0.0001$).

Correlations of the histological parameters with the bone morphological parameters showed that in tibial metaphyseal region, the Mankin total joint score negatively correlated with both the BMD ($r_s=-0.453$, $p=0.03$) and BV/TV ($r_s=-0.437$, $p=0.04$) in the MRL/MpJ strain, but did not correlate with any bone parameters in the C57BL/6 strain. The synovitis total joint score in the fractured limb positively correlated with both the BMD ($r_s=0.658$, $p=0.0001$) and BV/TV ($r_s=0.662$, $p=0.0001$) in the C57BL/6 strain, but not in the MRL/MpJ strain.

DISCUSSION:

The inflammation profiles of these two mice strains differ greatly, which may account for the difference in healing after articular fracture[11]. MRL/MpJ mice are reported to have increased levels of TGF-β_1, which may contribute to the enhanced bone response following fracture found in this study[12]. Previous reports have shown that C57BL/6 mice have elevated levels of pro-inflammatory cytokines IL-1 and TNF-α following joint injury[13]. An increased local inflammatory environment may contribute to altered bone morphology and subsequent degenerative changes in the joint tissues. The difference in these arthritic profiles indicates that there may be a benefit

to focusing first on fracture healing, then following up with suppression of the pro-inflammatory environment that leads to subsequent degradation of the joint.

Clinically, surgical restoration of the articular surface is the only treatment for articular fractures. To date, there is no method of identifying patients that are at risk for developing PTA. In addition to measures of joint pathology, serum and synovial fluid from both strains of mice will be analyzed for biomarkers. The comparison of imaging and biochemical biomarkers between mice and humans could improve the ability to detect the risk of PTA associated with the onset of an articular fracture.

SIGNIFICANCE:
By characterizing degenerative changes in the C57BL/6 and MRL/MpJ strains, key factors that contribute to the development of PTA can be identified. By understanding what drives disease progression, potential screening methods may be developed to identify patients at high risk of developing PTA.

FIGURES

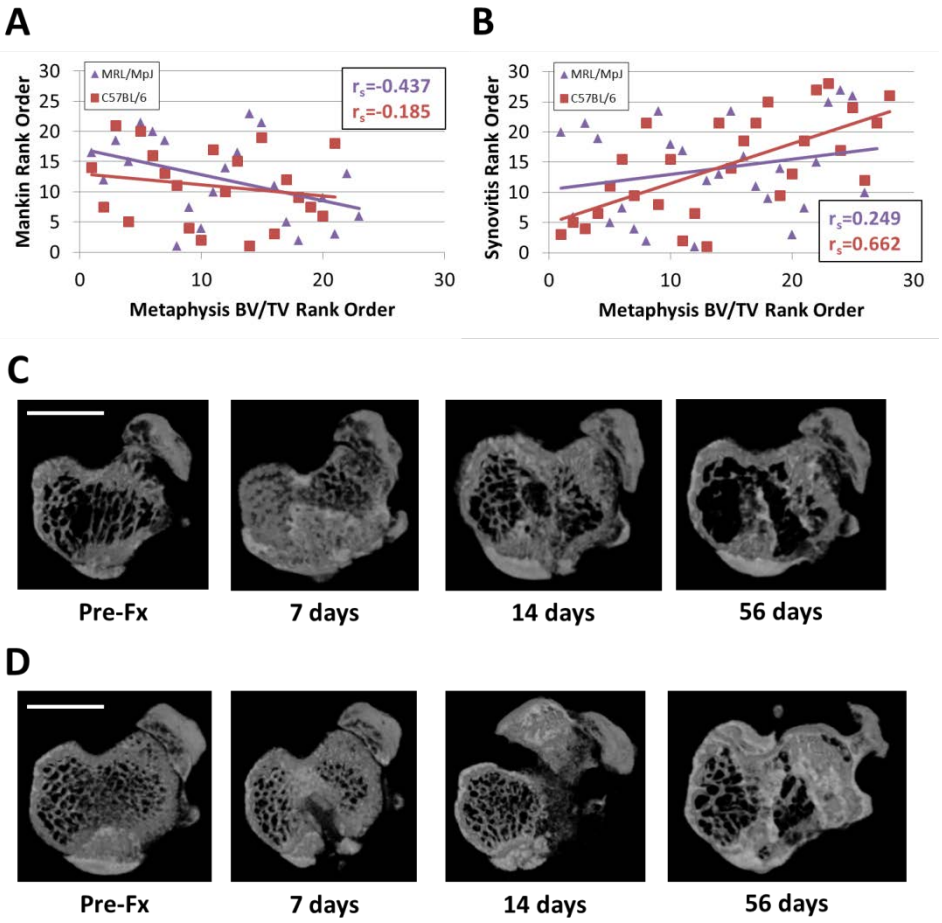


Figure 2. Correlations between Mankin total joint score, synovitis total joint score, and bone fraction (BV/TV) of the tibial metaphysis for both the MRL/MpJ and C57BL/6 strains. Values are displayed as a rank order within strain for each outcome measure (n=21-28 per strain). R_S values indicate Spearman correlation coefficient for each strain. **(A)** Mankin correlated with BV/TV (r_s=-0.437, p=0.0370) in the MRL/MpJ strain only. **(B)** Synovitis correlated with BV/TV (r_s=0.662, p=0.0001) in the C57BL/6 strain only. **(C)** Representative images of the tibial metaphysis for the C57BL/6 strain at each time point. Scale bar is 0.5mm. **(D)** Representative images of the tibial metaphysis for the MRL/MpJ strain at each time point. Scale bar is 0.5mm.

Novel In Vivo Micro-Computed Tomography Metrics of Joint Incongruity in the Mouse Knee Fracture Model

In vivo micro-CT scanning (SkyScan 1176, Bruker) of fractured limbs was performed before and after fracture, and then at 1, 4, and 8 weeks post-fracture. Bone surface deviations (BSD) were measured for all post-fx micro-CT scans. First, surface-rendered 3D digital models of the tibial plateau were generated from *in vivo* scans (CT-Analyser, Bruker). Models were then aligned to their respective pre-fx model's intact medial tibial plateau using an iterative closest point algorithm (Geomagic Studio, Geomagic®). BSDs were measured separately for the medial and lateral sides of the tibial plateau along three anatomic axes: antero-posterior (AP), latero-medial (LM), and axial (Geomagic Control, Geomagic®). Positive and negative deviations of the bone surface were measured (Fig 1), and defined as the distance to a test surface (post-fx bone surface) that was either outside (positive) or inside (negative) of the reference surface (pre-fx bone surface). A deviation of 0% corresponded to perfect alignment of pre-fx and post-fx tibial plateaus. Color maps of BSDs were generated for each anatomical direction. An example of axial deviations in an MRL/MpJ mouse from post-fx to 8 weeks is shown in Figure 3.

Temporal patterns in BSDs were significantly different between C57BL/6 and MRL/MpJ mice over 8 weeks (Figure 3). Significant differences were observed in the lateral tibial plateau as measured by average positive axial deviation ($p = 0.01$), average positive LM deviation ($p = 0.015$), and maximum positive LM deviation ($p = 0.01$). Additionally, a significantly larger average positive axial deviation was observed in C57BL/6 mice at 8 weeks post-fx ($p=0.01$) (Figure 4). In the medial plateau, significant differences were seen between strains as measured by average positive axial deviation ($p=0.049$) and average negative LM deviation ($p=0.049$).

DISCUSSION:

Through the development of novel *in vivo* micro-CT metrics of joint incongruity, we analyzed temporal patterns in bone surface deviations that revealed significant differences between C57BL/6 and MRL/MpJ strains over 8 weeks. Interestingly, after 8 weeks of fracture healing, we observed significantly larger bone surface deviations in C57BL/6 mice compared to MRL/MpJ mice.

SIGNIFICANCE:

In vivo micro-CT metrics of joint incongruity provide a method for quantifying bone surface incongruities that have traditionally been difficult to measure and provide new possibilities to guide PTA research and improve fracture management. The translational potential of our joint incongruity metrics is high, as they could readily be applied to full scale clinical CT scans.

FIGURES

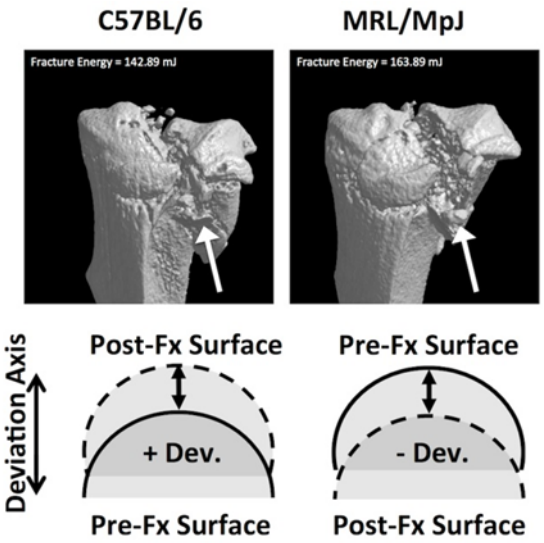


Figure 3. (Top) MicroCT images of representative fractures. (Bottom) Metrics of joint incongruity in the axial direction after fracture.

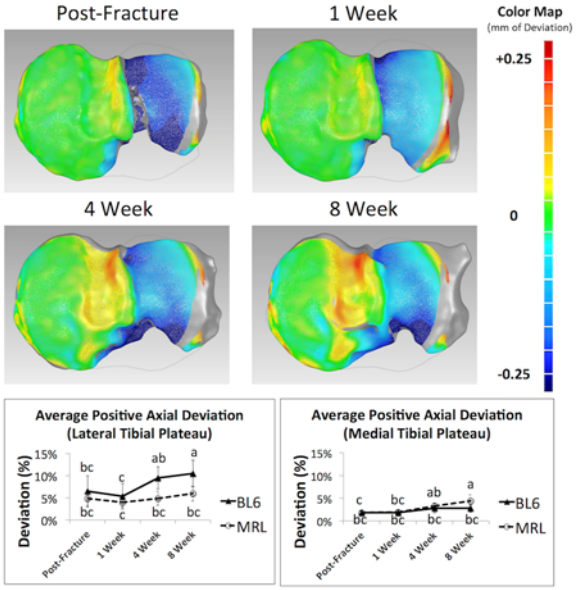


Figure 4. (Top) Representative color map of axial deviations with fracture healing. (Bottom) Significant strain-wise differences in fracture healing from immediately post-fracture to 8 weeks.

E. PROBLEM AREAS

Specific Aim 1:

The overall objective of this study is to identify biomarkers following articular fracture that may be predictive of the development of PTA. Specifically, patients with a closed unilateral articular fracture of the knee requiring operative treatment will be enrolled over an 18-month period. Biosamples (synovial fluid from the injured and contralateral uninjured knee, serum, and urine) will be collected prior to or at surgical intervention. MRI imaging of the injured knee will be obtained to assess the articular cartilage. Degenerative changes in the cartilage and joint space narrowing will be correlated to biomarkers that may be indicative and predictive of joint degeneration and the development of PTA. After expanding the enrollment criteria and extending the enrollment period to two years, enrollment has closed with 18 patients successfully enrolled in the study.

Issues:

As previously reported, enrollment was initially slow. However, in response, we expanded the enrollment criteria and extended the enrollment period. The enrollment has closed with 18 patients successfully enrolled in the study. Biosamples from all enrolled patients have been collected, processed and stored at -80°C. Questionnaires have also been collected from all patients. However, there have been issues with patient compliance in obtaining MRI scans, due to various factors including claustrophobia, definitive treatment outside the study criteria, such as conversion to total knee arthroplasty or only open reduction internal fixation, and noncompliance. We have obtained 11 post-op MRI scans, and 2 additional post-op scans are scheduled for the first week of November 2014. For 18-month follow-up MRI scans, we have obtained 2 scans, and 3 scans are scheduled for December 2014. We anticipate the remaining patients enrolled in the study to be compliant. Within the next reporting period, MRI scans will continue to be analyzed (Figure 1). In doing so, we will identify a subset of 6 patients that demonstrate evidence of degenerative arthritic changes in joint tissues and 6 patients with no evidence of degenerative arthritic changes. Proteomic analyses will be performed on these biosamples. Biomarker assays will be run on all collected biosamples. These results will be

compared to those obtained from the animal study in Specific Aim 2. However due to the slow initial enrollment, we anticipate that it will be necessary to request a no cost extension in order to complete all analyses.

Specific Aim 2: No problems/issues to report.

F. ANIMAL USAGE STATISTICS

- DOD Annual Report on Animal Use:
  - Species used: mice
  - Number of each species used: 84
- USDA Pain Category for all animals used:
  - Category C (Non-Painful Procedures): 12
  - Category D (Procedures using anesthesia/analgesia): 72

4. KEY ACCOMPLISHMENTS:

- Eighteen total patients have been enrolled in Aim 1. With 4 disqualified and 2 dropouts, the total is twelve, which is the target number of patients for statistical significance.
- Biosamples of serum, plasma, synovial fluid and urine have been collected, processed and stored from patients enrolled in Aim 1.
- All animals for Aim 2 were obtained and closed articular fractures of the tibial plateau were successfully created with a 100% success rate. Sacrifice and sample collection are complete for both the short-term and long-term arms of Aim 2.
- In vivo and ex vivo microCT analyses for Aim 2 are complete.
- Histologic analyses for the short-term arm of Aim 2 are complete. Histologic analyses for the long-term arm are in progress as of October 2014.

5. CONCLUSIONS:

Post-traumatic arthritis (PTA) is a severe burden in active duty and discharged soldiers. Recent figures from Operation Iraqi Freedom and Operation Enduring Freedom indicated joint degeneration following injury is the most common cause of a soldier being unfit for duty [3]. Compared to other forms of arthritis, (PTA) has a more rapid clinical onset [14]. This rapid onset of degenerative arthritis is occurring following joint injuries in a younger population of soldiers. The goal of this work is to identify biomarkers following articular fracture that may be predictive of the development of PTA. This knowledge is needed for future investigations to assess acute interventions to prevent PTA that can be given on the battlefield or at the time of stabilizing medical care in down range medical facilities. To reach this goal, the proposed investigation studies patients who have sustained a closed, displaced articular fracture about the knee requiring operative treatment. While these patients are not suffering battlefield injuries, they do represent significant articular injuries that are at risk for developing PTA. A companion murine bench top series of experiments will allow for the comparison of human and mouse response following joint fracture. An animal model is needed to allow for a low-cost model for the assessment of future therapies to prevent PTA.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS: Two abstracts describing the microCT analyses between the C57BL/6 and MRL/MpJ mice for the short-term and long-term cohorts from Aim 2 have been submitted for the annual Orthopaedic Research Society meeting in 2015.

7. INVENTIONS, PATENTS AND LICENSES: Nothing to report.

8. REPORTABLE OUTCOMES: The existing measures for clinical outcome are being applied in a novel manner to articular fractures. Data points such as VAS pain scores, KOOS, and SF-

36 have been completed by enrolled patients. The research is still in progress so reportable outcomes from the study are still pending.

9. OTHER ACHIEVEMENTS: None to report.

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11. APPENDICES: Nothing to report.